NTP REPORT ON CARCINOGENS BACKGROUND DOCUMENT for *p*-CHLORO-*o*-TOLUIDINE and ITS HYDROCHLORIDE SALT

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Carcinogenicity

p-Chloro-*o*-toluidine and its hydrochloride salt are *reasonably anticipated to be human carcinogens* based on limited evidence of carcinogenicity from studies in humans and evidence of malignant tumor formation in experimental animals (reviewed in IARC, 1990).

Evidence of *p*-chloro-*o*-toluidine carcinogenicity in humans is limited. Documented human exposure has occurred primarily in the dye and synthetic chemical industries. Between 1982 and 1990, seven cases of urinary bladder cancer were detected in a group of 49 workers producing the insecticide chlordimeform from *p*-chloro-*o*-toluidine on an irregular basis for an average of 18 years. The incidence of bladder tumors in this group was significantly higher than that of the cancer registers of the former German Democratic Republic, Saarland, and Denmark by 89.7-, 53.8-, and 35.0-fold, respectively. Exposure levels were not documented, but from 1980 to 1986, exposure to *p*-chloro-*o*-toluidine was analytically checked by monitoring of urine and was found to be minimal (quantitation of exposure not given). Increased incidences of tumors were observed primarily in the urinary bladder. One of the seven workers that had bladder cancer also developed a brain tumor. There was some evidence that the cohort studied handled other chemicals (including 4-chloroaniline); however, none of the resulting exposures were quantified by chemical analysis at the time (Popp et al., 1992). In other studies, workers were exposed to *p*-chloro-*o*-toluidine and numerous other compounds, several of which are known or possible carcinogens. Levels of exposure to all compounds were undocumented and occurred prior to the implementation of modern industrial hygiene standards in 1980 (Ott and Langer, 1983; cited by IARC, 1990; Stasik, 1988; Hogan, 1993).

A significant increase of hemangiosarcomas or hemangiomas was observed in both sexes of two strains of mice on chronic administration of *p*-chloro-*o*-toluidine hydrochloride in the diet. *p*-Chloro-*o*-toluidine hydrochloride was not a carcinogen when administered chronically in the diet of both sexes of two strains of rats (Weisburger et al., 1978; NCI, 1979).

Other Information Relating to Carcinogenesis or Possible Mechanism of Carcinogenesis

p-Chloro-*o*-toluidine has been demonstrated to be genotoxic in a variety of prokaryotic and mammalian *in vitro* and *in vivo* test systems (IARC, 1990). *p*-Chloro-*o*-toluidine binding to DNA was demonstrated *in vitro* with calf thymus DNA (Bentley et al., 1986) and *in vivo* when it was administered by intraperitoneal (i.p.) injection to rats (Hill et al., 1979; cited by IARC, 1990).

No data are available that would suggest that the mechanisms thought to account for tumor induction by *p*-chloro-*o*-toluidine in mice would not also operate in humans.

Listing Criteria from the Report on Carcinogens, Eighth Edition

Known To Be A Human Carcinogen:

There is sufficient evidence of carcinogenicity from studies in humans, which indicates a causal relationship between exposure to the agent, substance or mixture and human cancer.

Reasonably Anticipated To Be A Human Carcinogen:

There is limited evidence of carcinogenicity from studies in humans, which indicates that causal interpretation is credible but that alternative explanations, such as chance, bias, or confounding factors, could not adequately be excluded; or

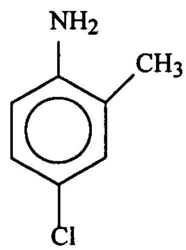
There is sufficient evidence of carcinogenicity from studies in experimental animals which indicates there is an increased incidence of malignant and/or a combination of malignant and benign tumors: (1) in multiple species or at multiple tissue sites, or (2) by multiple routes of exposure, or (3) to an unusual degree with regard to incidence, site or type of tumor, or age at onset; or

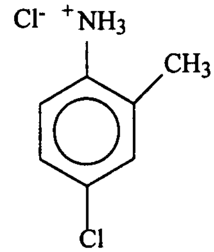
There is less than sufficient evidence of carcinogenicity in humans or laboratory animals; however, the agent, substance or mixture belongs to a well-defined, structurally related class of substances whose members are listed in a previous Report on Carcinogens as either a known to be human carcinogen or reasonably anticipated to be a human carcinogen, or there is convincing relevant information that the agent acts through mechanisms indicating it would likely cause cancer in humans.

Conclusions regarding carcinogenicity in humans or experimental animals are based on scientific judgement, with consideration given to all relevant information. Relevant information includes, but is not limited to dose response, route of exposure, chemical structure, metabolism, pharmacokinetics, sensitive sub populations, genetic effects, or other data relating to mechanism of action or factors that may be unique to a given substance. For example, there may be substances for which there is evidence of carcinogenicity in laboratory animals but there are compelling data indicating that the agent acts through mechanisms which do not operate in humans and would therefore not reasonably be anticipated to cause cancer in humans.

1.0 INTRODUCTION

p-Chloro-*o*-toluidine and Its Hydrochloride
[95-69-2 and 3165-93-3]





1.1 Chemical Identification of *p*-Chloro-*o*-toluidine

p-Chloro-*o*-toluidine (C_7H_8ClN, mol. wt. = 141.60) is also called:

Benzenamine, 4-chloro-2-methyl- (9Cl)
o-Toluidine, 4-chloro- (8Cl)
2-Amino-5-chlorotoluene
Azoic Diazo Component 11, base
Brentamine Fast Red TR Base
3-Chloro-6-aminotoluene
5-Chloro-2-aminotoluene
4-Chloro-2-methylaniline
4-Chloro-6-methylaniline
4-Choro-2-methylbenzenamine
4-Chloro-2-methylbenzeneamine
4-Chloro-2-toluidine
4-Chloro-*o*-toluidine
4-Chloro-*o*-toluidine (NH2=1)
Daito Red Base TR
Fast Red Base TR
Fast Red 5CT Base
Fast Red TR
Fast Red Tr11
Fast Red TR Base
Fast Red TRO Base
Kako Red TR Base
Kambamine Red TR
2-Methyl-4-chloroaniline
Mitsui Red TR Base
Red Base NTR
Red TR Base
Sanyo Fast Red TR Base
Tulabase Fast Red TR

1.2 Physical-Chemical Properties of *p*-Chloro-*o*-toluidine

Property	Information	Reference
Physical State	Leaflets	Weast (1985; cited by IARC, 1990)
Melting Point, °C	29-30	Weast (1985; cited by IARC, 1990)
Boiling Point, °C	241	Weast (1985; cited by IARC, 1990)
Solubility:		
Organic Solvents	Soluble in ethanol	Weast (1985; cited by IARC, 1990)

1.3 Chemical Identification of *p*-Chloro-*o*-toluidine Hydrochloride:

p-Chloro-*o*-toluidine hydrochloride ($C_7H_9Cl_2N$, mol. wt. = 178.07) is also called:

Benzenamine, 4-chloro-2-methyl-, hydrochloride (9Cl)	Daito Red Salt TR
	Devol Red K
o-Toluidine, 4-chloro-, hydrochloride (8Cl)	Devol Red TA Salt
Amarthol Fast Red TR Base	Devol Red TR
Amarthol Fast Red TR Salt	Diazo Fast Red TR
2-Amino-5-chlorotoluene hydrochloride	Diazo Fast Red TRA
Aniline, 4-chloro-2-methyl-, hydrochloride	Fast Red 5CT Salt
Azanil Red Salt TRD	Fast Red Salt TR
Azoene Fast Red TR Base	Fast Red Salt TRA
Azoene Fast Red TR Salt	Fast Red Salt TRN
Azogene Fast Red TR	Fast Red TR Salt
Brentamine Fast Red TR Salt	Hindasol Red TR Salt
Chlorhydrate de 4-chloroorthotoluidine (French)	Kromon Green B
5-Chloro-2-aminotoluene hydrochloride	2-Methyl-4-chloroaniline hydrochloride
4-Chloro-2-methylaniline hydrochloride	Natasol Fast Red TR Salt
4-Chloro-6-methylaniline hydrochloride	NCI-C02368
4-Chloro-2-methylbenzenamine hydrochloride	Neutrosel Red TRVA
4-Chlorotoluidine hydrochloride	Ofna-Perl Salt RRA
4-Chloro-2-toluidine hydrochloride	Red Base Ciba IX
4-Chloro-*o*-toluidine hydrochloride	Red Base Irga IX
p-Chloro-*o*-toluidine hydrochloride	Red Salt Ciba IX
4-Chloro-*o*-toluidine (NH2=1) hydrochloride	Red Salt Irga IX
C.I. 37085	Red TRS Salt
C.I. Azoic Diazo Component 11	Sanyo Fast Red Salt TR

1.4 Physical-Chemical Properties of *p*-Chloro-*o*-toluidine Hydrochloride

p-Chloro-*o*-toluidine hydrochloride's RCRA waste number is U049. Its UN shipping number is 1579. Available as a buff- or pink-colored powder (IARC, 1990), the compound is water-soluble.

1.5 Identification of Structural Analogues and Metabolites

Structural analogues and metabolites discussed in this report include the following:

5-Chloro-2-hydroxylaminotoluene
4-Chloro-2-methylphenylhydroxylamine (CMPHA)
4,4′-Dichloro-2,2'-dimethylazobenzene

No information was available regarding physical-chemical properties for the above compounds.

1.6 Report Organization

The remainder of this report includes six sections (2.0 Human Exposure, 3.0 Human Studies, 4.0 Mammalian Carcinogenicity, 5.0 Genotoxicity, 6.0 Other Relevant Data, 7.0 References) and two appendices. Appendix A describes the literature search in online databases, and Appendix B provides explanatory information for Figure 5-1.

2.0 HUMAN EXPOSURE

2.1 Use

p-Chloro-*o*-toluidine and its hydrochloride salt have been used commercially to produce azo dyes for cotton, silk, acetate, and nylon, and as intermediates in the production of Pigment Red 7 and Pigment Yellow 49. As an azoic diazo component, *p*-chloro-*o*-toluidine is used in the synthesis of some azoic dyes, which are made by a two-step process involving diazotization of a primary amine component and coupling of the diazotized amine with a naphthol-derived coupling component (IARC, 1990; NCI, 1979). *p*-Chloro-*o*-toluidine has also been used in the manufacture of the pesticide chlordimeform (IARC, 1990).

2.2 Production

Commercial production of *p*-chloro-*o*-toluidine began in Germany in 1924 and was first reported in the United States in 1939 (IARC, 1990). An IARC Working Group reported that production of *p*-chloro-*o*-toluidine in the United States stopped in 1979, and all importation and distribution was discontinued in 1986 (IARC, 1990). The USITC reported that between 1980 and 1983, the imports of *p*-chloro-*o*-toluidine and *p*-chloro-*o*-toluidine hydrochloride varied from a high of 89,753 pounds to a low of 31,747 (USITCa, 1981-1984). Chem Sources (1996) identified 11 U.S. suppliers of *p*-chloro-*o*-toluidine and four U.S. suppliers of *p*-chloro-*o*-toluidine hydrchloride.

2.3 Environmental Exposure

The routes of potential human exposure to *p*-chloro-*o*-toluidine and *p*-chloro-*o*-toluidine hydrochloride are inhalation, ingestion, and dermal contact.

2.3.1 Environmental Occurrence

p-Chloro-*o*-toluidine and *p*-chloro-*o*-toluidine hydrochoride are not known to occur naturally. *p*-Chloro-*o*-toluidine may be found in the environment as a decomposition product of chlordimeform. (See Subsection 2.3.2.)

2.3.2 Drinking Water and Food Content

p-Chloro-*o*-toluidine has been isolated and identified in field samples of plant materials treated with chlordimeform including bean leaves, grape stems, and fruits at levels ranging from 0.02 to 0.3 ppm. The compound was also reported to be formed from chlordimeform by enzymes present in the leaves of apple seedlings and in cotton plants (IARC, 1990).

In an experimental field application, residue concentrations of *p*-chloro-*o*-toluidine were found in rice grains at 3 to 61 ppb, in straw parts at 80 to 7200 ppb, in the upper 0 to 5 cm layer of soil at 2 to 68 ppb, and in the lower 5 to 10 cm of soil at trace concentrations to 20 ppb. In another experimental field application, residues of the compound were not detected in rice grains or husks (IARC, 1990).

2.3.3 Occupational Exposures

Occupations with the greatest potential for exposure include pigment manufacturers and dyemakers and manufacturers of chlordimeform. Exposures to *p*-chloro-*o*-toluidine have been reported to occur during the changing of mixing vats and at the basification stage in a purification plant in England, by inhalation and dermal contact at a batch-operated chemical processing plant in the United States, and during production and processing at a plant in the Federal Republic of Germany. Data on exposure levels were not provided for any of these studies (IARC, 1990).

p-Chloro-*o*-toluidine has been found in the urine of workers exposed to chlordimeform as its major metabolite (Popp et al., 1992; IARC, 1990). The National Occupational Hazard Survey, conducted by NIOSH from 1972 to 1974, estimated that 1397 workers were potentially exposed to *p*-chloro-*o*-toluidine in the workplace (NIOSH, 1976). The National Occupational Exposure Survey (1981-1983) indicated that 250 workers (all women) were potentially exposed to *p*-chloro-*o*-toluidine, and 682 workers, including 425 women, were potentially exposed to *p*-chloro-*o*-toluidine hydrochloride (NIOSH, 1984).

2.4 Regulations

EPA regulates *p*-chloro-*o*-toluidine hydrochloride under the Resource Conservation and Recovery Act (RCRA), Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA), Superfund Amendments and Reauthorization Act (SARA), and the Toxic Substances Control Act (TSCA). *p*-Chloro-*o*-toluidine is regulated under TSCA. EPA has established rules for regulating hazardous spills and requirements for handling and disposal of wastes. *p*-Chloro-*o*-toluidine hydrochloride is regulated as a hazardous constituent of waste under RCRA and is subject to report/recordkeeping requirements under RCRA and SARA. A statutory reportable quantity (RQ) of one lb (0.454 kg) was established for *p*-chloro-*o*-toluidine hydrochloride, but EPA increased the RQ to 100 lb (45.4 kg) under CERCLA. TSCA subjects both compounds to reporting requirements applicable to any significant new use. The Department of Transportation (DOT) has its own regulations concerning the transportation of *p*-chloro-*o*-toluidine. OSHA regulates *p*-chloro-*o*-toluidine and *p*-chloro-*o*-toluidine hydrochloride under the Hazard Communication Standard and as a chemical hazard in laboratories.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 148.15(d). Effective 08/08/90. RCRA 3004: Hazardous Waste Injection Restrictions. Waste Specific Prohibitions—Second Third Wastes.	Chemicals listed in, but not limited to 40 CFR 261.33, are prohibited from underground injection at off-site injection facilities.
	40 CFR 148.15(f). Effective 11/08/90. RCRA 3004: Hazardous Waste Injection Restrictions. Waste Specific Prohibitions—Second Third Wastes.	Chemicals listed in, but not limited to, 40 CFR 261.33 are prohibited from underground injection at on-site injection facilities.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 261.11, 261.33. Promulgated 5/19/80. RCRA: Identification and Listing of Hazardous Waste. Designates *p*-chloro-*o*-toluidine hydrochloride as a hazardous waste subject to recordkeeping and reporting requirements.	*p*-Chloro-*o*-toluidine hydrochloride has been identified as a primary hazardous material (U049) by its toxicity and is regulated under the hazardous waste disposal rule of RCRA.
	40 CFR 261.33(f). Promulgated 07/01/90. RCRA 3010: Final rule for discarded commercial chemical products, off-specification species, container residues, and spill residues.	Chemical class U wastes and toxic wastes.
	40 CFR 268.11. Promulgated 5/28/86. RCRA 3004: Land Disposal Restrictions. Schedule for land disposal prohibition and establishment of treatment standards. Identifies restricted wastes and concentrations of their hazardous constituents which may not be exceeded.	Restrictions or prohibitions for storage and land disposal of *p*-chloro-*o*-toluidine hydrochloride to be evaluated by June 8, 1989.
	40 CFR 268.35(a). Effective 01/31/91. RCRA 3004: Technical amendment to the final rule for effective dates of surface disposed wastes (non-soil and debris) regulated in the Land Disposal Restrictions—Comprehensive List.	Waste specific prohibitions—Third Third wastes. Effective date of prohibition from land disposal was 08/08/90.
	40 CFR 268.35(d). Effective 01/31/91. Revised at 57 FR 47776, 10/20/92. RCRA 3004: Technical amendment to the final rule for effective dates of surface disposed wastes (non-soil and debris) regulated in the Land Disposal Restrictions for mixed radioactive/ hazardous wastes.	Effective date of prohibition from land disposal was 05/08/92. *p*-Chloro-*o*-toluidine hydrochloride is the hazardous component of mixed radioactive/ hazardous wastes.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 268.42, Table 2. Effective 01/31/91. Amended through 05/24/93. RCRA: Treatment Standards Expressed as Specific Technologies.	Listing of Technology-Based Standards by RCRA waste code in wastewater as wet air oxidation or chemical/electrolytic oxidation followed by carbon absorption or incineration. Nonwastewater is fuel incineration only.
	40 CFR 268, Appendix IV. Effective 01/31/91. RCRA: Land Disposal Restrictions on Organometallic Lab Packs.	Lists hazardous waste by the EPA hazardous waste code number (U049) for disposal in an organometallic lab pack.
	40 CFR 268, Appendix V. Effective 01/31/91. RCRA: Land Disposal Restrictions on Organic Lab Packs.	Lists hazardous waste by the EPA hazardous waste code number (U049) for disposal in an organic lab pack.
	40 CFR 268, Appendix VII, Table 1. Effective 01/31/91. RCRA: Land Disposal Restrictions, Comprehensive List.	Comprehensive listing of the effective dates of surface disposal wastes (non-soil and debris) regulated in the Land Disposal Restrictions (LDRs) and listed by the EPA hazardous waste code number. U049 has an effective date of 08/08/90 in all waste categories.
	40 CFR 302.4, Table 302.4. Promulgated 8/14/89. CERCLA 102(a): List of hazardous substances and reportable quantities.	Final rule established RQ of 100 lb (45.4 kg) for *p*-chloro-*o*-toluidine hydrochloride under CERCLA 102(a) when RCRA 3001 established the RQ of 1 lb (0.454 kg).
	40 CFR 372.65. Proposed rule 59 FR 1788 01/12/94. SARA 313: Toxic Chemical Release Inventory Reporting under Community Right-to-Know. Proposed rule to add 313 chemicals and chemical categories (including *p*-chloro-*o*-toluidine) to the list of toxic chemicals required to be reported on under section 313 of the Emergency Planning and Community Right-to-Know Act.	Would require public notice of the release of a toxic chemical and also require suppliers to notify persons to whom they distribute of the presence of these toxic chemicals in their products. Comments on this proposed rule were to have been received by 04/12/94.

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
E P A	40 CFR 721.462. Promulgated 07/01/90. TSCA 5(a)(2): Significant New Use Rule (SNUR).	Establishes procedures for the reporting of new chemical substances and defines the persons and chemical substances subject to the reporting requirements.
O S H A	29 CFR 1910.1200. Promulgated 2/15/89. OSH Act: Hazard Communication Standard.	Requires chemical manufacturers and importers and all employers to assess chemical hazards and to provide information to employees. Hazard Communication program to include labels, material safety data sheets, and worker training.
	29 CFR 1910.1450. Promulgated 1/31/90. Amended 55 FR 12111, 3/30/90. OSH Act: Final rule for occupational exposure to hazardous chemicals in laboratories.	As select carcinogens (IARC Group 2A), *p*-chloro-*o*-toluidine and *p*-chloro-*o*-toluidine hydrochloride are included as chemical hazards in laboratories. Employers are required to provide employee information and training and to provide Chemical Hygiene Plan.
D O T	49 CFR 172.101. Effective 10/01/91. DOT: Hazardous Materials Table.	The DOT classifies the materials within as hazardous for the purpose of transportation, setting requirements for the packaging, labeling, and quantity limits aboard passenger aircraft or railcar as 100 kg and cargo aircraft only as 200 kg. In addition, quantity limitations are also set for stowage aboard vessels.
	49 CFR 172.101, Appendix A, Table 1. Effective 12/31/91. DOT: List of Hazardous Substances and Reportable Quantities.	Lists materials which are considered hazardous substances under CERCLA with their corresponding reportable quantities (RQs). For *p*-chloro-*o*-toluidine hydrochloride the RQ is 100 lb (45.4 kg).

REGULATIONS

	Regulatory Action	Effect of Regulation/Other Comments
D O T	49 CFR 172.102. Promulgated 10/01/90. DOT: Special Provisions for Transportation.	Special provisions are given for packaging, labeling, and transportation of hazardous materials in Table 172.101. St. Andrew's Cross label required for *p*-chloro-*o*-toluidine hydrochloride.

3.0 HUMAN STUDIES

Summary: There is "limited evidence" for the carcinogenicity of *p*-chloro-*o*-toluidine in humans (IARC, 1990). All data for human studies come from evaluations of chemical workers who have handled *p*-chloro-*o*-toluidine in the workplace and who were also potentially exposed to other chemicals, some of them suspected or known carcinogens. In the studies that have reported or suggested that *p*-chloro-*o*-toluidine is a human carcinogen, the target organ was the urinary bladder. Although no studies were found that evaluated the human carcinogenicity of *p*-chloro-*o*-toluidine hydrochloride, it is expected that this compound has a similar chemical behavior to *p*-chloro-*o*-toluidine in mammalian systems (IARC, 1990).

Gross hematuria and strangury (slow and painful urine discharge) were exhibited in 11 workers exposed to *p*-chloro-*o*-toluidine in the United Kingdom. Most had suprapubic pain and all displayed symptoms within days of initial exposure. Follow-up examination of three of these patients within three years of the onset of illness revealed that one had no further bladder trouble, one had slight cystitis and urethritis, and one had a carcinoma of the bladder. The dose and duration of exposure were not given and it was not specified whether the workers were also exposed to other chemicals (Currie, 1933; cited by IARC, 1990).

A cohort study of 342 men who manufactured dyes in the United States between 1914 and 1958 was conducted by Ott and Langer (1983; cited by IARC, 1990). Of the 342 men, 117 were involved in brom- and thioindigo production which potentially exposed them to *p*-chloro-*o*-toluidine and other raw materials and intermediates, including *o*-toluidine. Follow-up examination of these 117 men from 1940 to 1975 revealed a nonsignificant excess of cancer deaths (12.0 observed, 8.0 expected from age-specific U.S. white mortality rates) and no bladder cancer (expected figure unspecified, but estimated to be ~0.5).

Stasik (1988) followed up on a historical mortality study (Stasik et al., 1985, a U.S. Environmental Protection Agency [EPA] TSCA 8(e) submission by American Hoechst Corporation) of 335 male workers involved in the processing and production of *p*-chloro-*o*-toluidine. In the earlier study (Stasik et al., 1985), no deaths from cancer of the urinary bladder were reported, but after the completion of this earlier study, Stasik (1988) noted the occurrence of eight cases of urinary bladder cancer, with two deaths occurring by December 1986. The eight affected men were part of a subcohort of 116 male workers who had begun employment before 1970 when improvements in industrial hygiene were implemented. Prior to 1970, the men were exposed to higher levels of monocyclic arylamines, *p*-chloro-*o*-toluidine, and *N*-acetyl-*o*-toluidine. Although the exact level of exposure to these chemicals was not known, analysis of the production process indicated that exposure to *p*-chloro-*o*-toluidine was "considerably higher" than to *N*-acetyl-*o*-toluidine. The eight men were exposed for a median of 14.0 years before 1970, and a median total of 25.5 years before and after 1970. The median age at the beginning of exposure was 35.5 years, and the median age at diagnosis of bladder cancer was 64.0 years with a latency period of 27.5 years. Stasik et al. (1985; cited by Stasik, 1988) calculated that ~0.5 deaths from malignant neoplasms of the urogenital tract were expected in the subcohort of 116 workers who had begun employment prior to 1970.

The Schering Corporation (1989) reported in a risk-notification letter to the U.S. EPA that four of its workers in West Germany who had handled *p*-chloro-*o*-toluidine from 1968 to 1976 during manufacture of the insecticide chlordimeform had developed bladder tumors. They also noted that these workers were occupationally exposed to a number of other chemicals (not specified).

Hogan et al. (1993) re-examined a study conducted by Ward et al. (1988; cited by Hogan, 1993) that had reported noninvasive papillary tumors in three former employees of a batch chemical plant that manufactured 4,4′-methylenebis(2-chloroaniline) (MBOCA) in the 1970s. Hogan et al. (1993) reported that the three workers had been potentially exposed not only to MBOCA, but also to polybrominated biphenyls, aniline, *o*-toluidine, 4,4′-methylenedianiline, and *p*-chloro-*o*-toluidine. The dose and duration of exposure to *p*-chloro-*o*-toluidine were not specified.

Popp et al. (1992) studied the increased incidence of bladder cancer in a cohort of workers exposed to *p*-chloro-*o*-toluidine while producing chlordimeform in a German chemical plant (thought to be the same chemical plant the Schering Corporation reported on above). This cohort of 49 males was subjected to different periods of exposure from 1965 to 1976 and 1980 to 1986 due to sporadic production of chlordimeform. From 1980 to1986, exposure to *p*-chloro-*o*-toluidine was analytically checked by monitoring of urine and was found to be minimal.

The period of investigation began with the entry of the subject into the company's employment (1950-59, n=17; 1960-69, n=4; 1970-79, n=28) and ended with the detection of bladder cancer (n=7), premature termination of employment (n=8), death from other causes (n=2), or the end of the year 1990. Thirty-nine subjects remained at the end of this study (end of 1990), with an average of 18 years of sporadic exposure to *p*-chloro-*o*-toluidine documented. The individual cumulative exposure ranged from 3 to 956 days. The standard incidence rates (SIR; the ratio of the number of cases observed [O] to the expected number [E]) of bladder carcinoma in this group of workers were determined to be 89.7-, 53.8-, and 35-fold higher in the cohort studied, depending on which cancer register (GDR, Saarland, and Denmark, respectively) to which they were compared (see Table 3-2). The p values ranged between 0.000002 and 0.00001. None of the workers who were handling only the final product chlordimeform at the formulation or packing plants developed bladder cancer by the end of 1990. The results of this study are presented in Tables 3-1 and 3-2.

There was some evidence that the cohort studied handled other chemicals at the German chemical plant; however, none of the resulting exposures were quantified by chemical analysis at the time.

In five of the seven bladder cancer patients, the acetylator phenotype was determined (slow, n=4; fast, n=1). "This agrees with other studies reporting an increased risk of bladder tumors caused by arylamines in slow acetylators" (Weber and Hein, 1985; Lewalter and

Miksche, 1991; both cited by Popp et al., 1992). The authors also stated that the exposure (average, 575 days; see Table 3-1) and latency times (average, 19 years; see Table 3-1) are in agreement with the results of Stasik (1988) and Stasik (1991; cited by Popp et al., 1992).

Table 3-1. Smoking habits, times of exposure and latency, age at diagnosis, and acetylator phenotype in seven workers with bladder cancer. Recreated from Popp et al. (1992).

Subject	Diagnosis	Smoking Habit*	Exposure	Latency (yr)	Age at diagnosis (yr)	Acetylator Phenotype
1	Transitional cell carcinoma	Non-smoker	Sporadic before 1976	21	57	Deceased
2	Transitional cell carcinoma	10-a-day	291 days (1966-71)	23	47	
3	Papillary carcinoma	Non-smoker	555 days (1968-75)	16	48	Slow
4	Transitional cell carcinoma	10-a-day	617 days (1968-76)	17	62	Slow
5	Transitional cell carcinoma	Non-smoker	766 days (1968-76)	21	62	Slow
6	Transitional cell carcinoma	20-a-day	644 days (1968-76)	15	43	Slow
7	Transitional cell carcinoma	20-a-day	Sporadic (1966-74)	17	56	Fast
Average			**575 days[b]**	**19**	**54**	

*Number of cigarettes
[b]Without subjects 1 and 7

Table 3-2. Standard incidence rates of bladder carcinoma in the general population compared to the incidence in the group of workers (n = 49) engaged in chlordimeform synthesis. Recreated from Popp et al. (1992).

Cases observed (O)	E (Country)	SIR	95% Confidence Interval	*p* Value
7	0.078 (GDR)	89.7	35.6-168.6	0.000002
7	0.200 (Denmark)	35.0	13.9-65.7	0.00001
7	0.130 (Saarland)	53.8	21.3-101.1	0.000005

E = expected incidence in 49 workers over 18 years based on incidence in general population
SIR (Standard Incidence Rate) = actual bladder tumor incidence in 49 workers over an average of 18 years divided by the expected number

4.0 MAMMALIAN CARCINOGENICITY

Full experimental details for the studies described in this section are presented in Tables 4-1 and 4-2.

Summary: In an 8(e) submission by Ciba Geigy (1974a,b) to the U.S. EPA under the Toxic Substances Control Act, it was reported that there was an increase in the incidences of subcutaneous and abdominal "unclassified malignant tumors" in ICR mice administered *p*-chloro-*o*-toluidine in the diet for 80 weeks and that there was an increase in the incidence of hepatoma, benign and malignant, in male and female Sprague-Dawley rats administered *p*-chloro-*o*-toluidine in the diet for 80 weeks. Doses ranged from 20 to 500 ppm. There was no mention of statistical analysis of tumor incidence. The lack of statistical analysis and poor survival of animals at doses that were lower than those administered in other studies described in this section are indicative of an inadequate study.

There is "sufficient evidence" for the carcinogenicity of *p*-chloro-*o*-toluidine hydrochloride in experimental animals (IARC, 1990). Vascular tumor (hemangiosarcoma or hemangioma) incidence increased in male and female CD-1 albino mice administered *p*-chloro-*o*-toluidine hydrochloride in the diet (males: 750 or 1500 ppm; females: 2000 or 4000 ppm) for 18 months and in male and female B6C3F$_1$ mice administered *p*-chloro-*o*-toluidine hydrochloride in the diet (males: 15000 ppm; females: 1250 or 5000 ppm) for up to 99 weeks.

There was no significant increase in the incidence of tumors of the major organs in male Sprague-Dawley rats (females not evaluated) administered up to 4000 ppm *p*-chloro-*o*-toluidine hydrochloride in the diet for 3 months, followed immediately with up to 1000 ppm *p*-chloro-*o*-toluidine hydrochloride for an additional 15 months. However, this study was inadequate.

4.1 *p*-Chloro-*o*-toluidine

4.1.1 Mice

In an 8(e) submission by Ciba Geigy (1974a) to the U.S. EPA under the Toxic Substances Control Act, an increase was reported in the incidence of subcutaneous "unclassified malignant tumors" in male and female ICR mice (age not specified) administered 500 ppm *p*-chloro-*o*-toluidine in the diet for up to 80 weeks. The incidence of these tumors was increased, however, only in mice that died before the end of the treatment period. In addition, these tumors were not detected in mice administered 20 or 100 ppm *p*-chloro-*o*-toluidine in the diet for up to 80 weeks. The incidence of "unclassified malignant tumors" of the abdominal cavity was also increased in male mice fed 100 or 500 ppm and in female mice fed 20, 100, or 500 ppm *p*-chloro-*o*-toluidine in the diet for up to 80 weeks. Again, these incidences were only increased in mice that died before the end of the treatment period. There was no mention of statistical analysis of tumor incidence.

4.1.2 Rats

In an 8(e) submission by Ciba Geigy (1974b) under the Toxic Substances Control Act, it was reported that there was an increased incidence of malignant hepatoma in male and female Sprague-Dawley rats (age not specified) administered 20, 100, or 500 ppm *p*-chloro-*o*-toluidine in the diet for 80 weeks and of "probably benign hepatoma" in rats fed 100 or 500 ppm *p*-chloro-*o*-toluidine in the diet for 80 weeks. In rats that died before the end of the treatment period, the incidence of malignant hepatoma was increased in males and females fed 500 ppm, the incidence of "probably malignant hepatoma" was increased in males fed 20, 100, or 500 ppm and in females fed 500 ppm, and the incidence of "probably benign hepatoma" was increased in males fed 500 ppm and in females fed 100 or 500 ppm. There was no mention of statistical analysis of tumor incidence.

4.2 *p*-Chloro-*o*-toluidine Hydrochloride

4.2.1 Mice

The incidence of vascular tumors (hemangiosarcomas or hemangiomas, found mainly in the spleen and subcutaneous and retroperitoneal adipose tissues) was significantly increased in male and female CD-1 albino mice that were administered *p*-chloro-*o*-toluidine hydrochloride in the diet (males: 750 or 1500 ppm; females: 2000 or 4000 ppm) for 18 months starting at age 6 to 8 weeks. No other statistically significant neoplasms were detected in a number of other tissues (Weisburger et al., 1978).

The incidence of hemangiosarcoma (originating in the fatty tissue adjacent to the genital organs, but also sometimes infiltrating to abdominal muscles, uterus, ovaries, prostate gland, or urinary bladder) was increased in female B6C3F$_1$ mice that were administered *p*-chloro-*o*-toluidine hydrochloride (1250 or 5000 ppm in the diet) for 92 (high-dose females) or 99 (low-dose females) weeks from age 6 weeks and in male B6C3F$_1$ mice that were administered 15,000 ppm in the diet for 99 weeks starting at age 6 weeks. The incidence of hemangiosarcoma in male mice administered 3750 ppm in the diet was not significantly increased as compared to untreated controls. No other statistically significant neoplasms were detected in a number of other tissues (NCI, 1979).

4.2.2 Rats

There was no significant increase in the incidence of tumors in male Charles River CD Sprague-Dawley-derived rats, 6- to 8-weeks-old at treatment initiation, administered *p*-chloro-*o*-toluidine hydrochloride in the diet (2000 or 4000 mg/kg diet for 3 months, followed immediately by 500 or 1000 mg/kg diet [2808 or 5616 μmol/kg] for an additional 15 months). The administered dose was lowered after 3 months either because of low weight gain or because of chemically induced deaths (it was not specified which was the actual cause). However, it was suggested that the maximally tolerated dose (MTD) had been reached. All grossly abnormal organs, tumors masses, lungs, liver, spleen, kidneys, adrenal glands, heart, bladder, stomach, intestines, reproductive organs, and pituitary gland were histologically examined (Weisburger et al., 1978). Because the study was terminated at 18 months, because no necropsy was conducted in the early deaths, because no mortality adjusted statistics were used, and because the groups of treated and control animals were small, a positive effect may have been obscured.

The incidence of chromophobe adenoma of the pituitary gland was significantly increased in female, but not male, Fischer 344 rats administered 1250 or 5000 mg *p*-chloro-*o*-toluidine hydrochloride per kilogram diet (7020 or 28,080 μmol/kg) for 107 weeks, beginning at age 6 weeks. This increase, however, was not considered to be biologically significant because of the reduced survival in the control group and the abnormally low concurrent control rate of pituitary gland tumors (5%) relative to historical rates (21%). No other statistically significant neoplasms were detected in a number of other tissues (NCI, 1979).

Table 4-1. Mammalian Carcinogenicity of *p*-Chloro-*o*-toluidine

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice							
mice (age at initiation of study not given)	30M, 30F per dose	30M, 30F (untreated)	*p*-chloro-*o*-toluidine, purity not specified	20, 100, or 500 ppm in diet	80 wk	Surviving mice were killed at the end of the treatment period. All HD females died by 67 weeks. Only 1 HD male survived at 80 weeks. The following tissues were examined: heart, lungs, spleen, liver, kidneys, stomach, small intestine, testes, ovaries, adrenal glands, pancreas, eyes, pituitary gland, thyroid gland, thymus gland, lymph nodes, urinary bladder, bone marrow, brain, and peripheral nerve. There was no mention of statistical analysis of tumor incidence, but it was reported that "there was an increase in the incidence of 'unclassified malignant tumor' subcutaneous and in the abdominal cavity of 20, 100, and/or 500 ppm mice. **Subcutaneous Tissue:** "Unclassified malignant tumors" were detected in 12/28 HD males and 10/28 HD females that died before the end of the treatment period (vs. 0/9 male controls and 0/13 female controls). These tumors were not detected in LD and MD mice that died before the end of the treatment period. **Abdominal Cavity:** The incidence of unclassified malignant tumors was increased in treated mice (0/11 LD, 9/20 MD, and 10/28 HD males vs. 0/9 controls; 10/19 LD, 10/24 MD, and 10/28 HD females, vs. 0/13 controls). There were no significant abdominal cavity tumors detected in treated mice that were sacrificed at the end of the treatment period. **Other Tissues:** There was no increase in the incidence of tumors in other tissues of treated mice.	Ciba-Geigy (1974a)

Table 4-1. Mammalian Carcinogenicity of *p*-Chloro-*o*-toluidine (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats							
rats (age at initiation of study not given)	30M, 30F per dose	30M, 30F	*p*-chloro-*o*-toluidine, purity not specified	20, 100, or 500 ppm in diet	94 wk (males) 104 wk (females)	Surviving rats were killed at the end of the treatment period. The following tissues were examined: heart, lungs, spleen, liver, kidneys, stomach, small intestine, testes, ovaries, adrenal glands, pancreas, eyes, pituitary gland, thyroid gland, thymus gland, lymph nodes, urinary bladder, bone marrow, brain, and peripheral nerve. There was no mention of statistical analysis of tumor incidence, but it was reported that "histologic evaluation of tissue revealed an increased incidence of liver tumors, benign and malignant. **Liver:** Malignant hepatoma was detected in 1/9 HD males; 1/10 MD females, and 3/6 HD females that were sacrificed at the end of the treatment period. Two of 9 HD males that were sacrificed at the end of the treatment period were diagnosed with "probably malignant hepatoma". "Probably benign" hepatoma was detected in 1/6 MD and 4/9 HD males (vs. 0/13 controls) and in 4/10 MD and 1/6 HD females (vs. 0/14 controls) that were sacrificed at the end of the treatment period. Malignant hepatoma was detected in 1/21 HD males and 1/24 HD females (vs. 0/17 male controls and 0/15 female controls) that died before the end of the treatment period. These tumors were not detected in LD or MD rats that died before the end of the treatment period. "Probably malignant hepatoma" was detected in 1/20 LD, 1/24 MD, and 1/21 HD males (vs. 0/17 controls) and in 1/24 HD females (vs. 0/15 controls) that died before the end of the treatment period. "Probably benign hepatoma" was detected in 3/21 HD males (vs. 0/17 controls) and in 1/19 MD and 12/24 HD females (vs. 0/15 controls) that died before the end of the treatment period. **Other Tissues:** There was no increase in the incidence of tumors in other tissues of treated mice.	Ciba-Geigy (1974b)

Abbreviations: F = females; HD = high dose; LD = low dose; M = males; MD = mid dose

Table 4-2. Mammalian Carcinogenicity of *p*-Chloro-*o*-toluidine Hydrochloride

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice							
6- to 8-wk-old CD-1 albino mice (derived from HaM/ICR mice)	25M, 25F per dose	25M, 25F (untreated)	*p*-chloro-*o*-toluidine HCl, 97-99% pure	*males*: 750 or 1500 ppm in diet *females*: 2000 or 4000 ppm in diet	18 mo	Mice were killed 3 months after the end of treatment. Mice that died during the first 6 months of treatment were not necropsied. Gross necropsy was performed on mice that died or were killed after 6 or more months of treatment. The following tissues were examined histologically: all gross lesions, tumor masses, lungs, liver, spleen, kidneys, adrenal glands, heart, bladder, stomach, intestines, and reproductive organs. The Fisher exact test was used for statistical analysis of tumor incidence. Tumors in treated animals with p-values of ≤ 0.05 for both matched and pooled controls were considered statistically significant. **Vascular System:** Positive (for hemangiosarcoma or hemangioma) The incidence of vascular tumors (hemangiosarcomas or hemangiomas, found mainly in the spleen and subcutaneous and subperitoneal adipose tissues) was significantly increased in treated mice (12/20 LD males, 13/20 HD males, 18/19 LD females, and 12/16 HD females vs. none in controls [$p < 0.025$]). In pooled controls from a larger study of several compounds, these tumors were detected in 5/99 males and 9/102 females. A breakdown of the incidences of hemangiosarcoma and hemangioma was not given.	Weisburger et al. (1978)

Table 4-2. Mammalian Carcinogenicity of *p*-Chloro-*o*-toluidine Hydrochloride (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
6-wk-old B6C3F$_1$ mice	50M, 50F (each dose level)	20M, 20F (untreated)	*p*-chloro-*o*-toluidine HCl, >99% pure	*males*: 3750 or 15000 ppm in diet *females*: 1250 or 5000 ppm in diet	92 wk (HD females) 99 wk (all other mice)	All HD females died by 92 weeks. Mice were killed at the end of the treatment period. The mean body weights of *p*-chloro-*o*-toluidine HCl-treated mice were lower than those of corresponding controls in a dose-related manner (significance not specified). Major tissues and organs were examined macroscopically for lesions. Microscopic examinations were performed on skin, lungs and bronchi, trachea, bone marrow (femur), spleen, lymph nodes (mesenteric and submandibular), thymus, heart, salivary glands (parotid, sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and nonglandular), small and large intestine, kidneys, urinary bladder, pituitary, adrenal glands, thyroid gland, parathyroid gland, testes, prostate gland, mammary glands, uterus, ovaries, brain (cerebrum and cerebellum), and all tissue masses. The Fisher exact test and Cochran-Armitage test were used for statistical analyses of tumor incidence. **Vascular System:** Positive (for hemangiosarcoma; HD males and LD and HD females) Hemangiosarcomas (originating in the fatty tissue adjacent to the genital organs, but also sometimes infiltrating to abdominal muscles, uterus, ovaries, prostate, or urinary bladder) were detected in 37/50 HD males [$p < 0.001$], 40/49 LD females [$p < 0.001$], and 39/50 HD females [$p < 0.001$] (vs. none in controls). **Other:** No other statistically significant neoplasms were detected in other organs.	NCI (1979)
Rats							
6- to 8-wk-old Charles River CD Sprague-Dawley-derived rats	25M (for each of 2 doses)	25M (untreated)	*p*-chloro-*o*-toluidine HCl, 97-99% pure	2000 or 4000 ppm in diet for 3 mo, followed immediately by 500 or 1000 ppm in diet for an additional 15 mo	18 mo	Rats were killed 6 months after the end of treatment. Rats that died during the first 6 months of treatment were not necropsied. Gross necropsy was performed on rats that died or were killed after 6 or more months of treatment. The following tissues were examined histologically: all gross lesions, tumor masses, lungs, liver, spleen, kidneys, adrenal glands, heart, bladder, stomach, intestines, reproductive organs, and pituitary gland. The Fisher exact test was used for statistical analysis of tumor incidence. Tumors in treated animals with p-values of ≤ 0.05 for both matched and pooled controls were considered statistically significant. **All Examined Tissues:** Negative Tumor incidence did not differ significantly between treated and control rats.	Weisburger et al. (1978)

Table 4-2. Mammalian Carcinogenicity of *p*-Chloro-*o*-toluidine Hydrochloride (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
6-wk-old Fischer 344 rats	50M, 50F (per dose level)	20M, 20F (untreated)	*p*-chloro-*o*-toluidine HCl, >99% pure	1250 or 5000 ppm in diet	107 wk	Groups of 50 males and 50 females were initially treated and 20 males and 20 females were initially used as controls. Rats were killed at the end of the treatment period. The mean body weights of the HD males and females were lower than the corresponding controls (significance not specified). Treated rats lived longer than controls (significance not specified). Major tissues and organs were examined macroscopically for lesions. Microscopic examinations were performed on skin, lungs and bronchi, trachea, bone marrow (femur), spleen, lymph nodes (mesenteric and submandibular), thymus, heart, salivary glands (parotid, sublingual, and submaxillary), liver, pancreas, esophagus, stomach (glandular and nonglandular), small and large intestine, kidneys, urinary bladder, pituitary gland, adrenal glands, thyroid gland, parathyroid gland, testes, prostate gland, mammary glands, uterus, ovaries, brain (cerebrum and cerebellum), and all tissue masses. The Fisher exact test was used for statistical analysis of tumor incidence. **Pituitary Gland:** The incidence of chromophobe adenoma was significantly increased in dosed females (13/48 LD [$p = 0.039$], 15/48 HD [$p = 0.020$] vs. 1/19 controls). This increase, however, was not considered to be biologically significant because of the reduced survival in the control group, and the abnormally low concurrent control rate of pituitary gland tumors (5%) relative to historical control rates (21%) for females. **Other:** No other statistically significant neoplasms were detected in other organs.	NCI (1979)

Abbreviations: F = females; HD = high dose; LD = low dose; M = males; MD = mid dose

5.0 GENOTOXICITY

Studies of the genotoxic effects of *p*-chloro-*o*-toluidine are summarized in Table 5-1.

Summary: A variety of prokaryotic and mammalian *in vitro* and *in vivo* test systems [see Genetic Activity Profile, Figure 5-1 (Data limited to IARC, 1990)] provided clear evidence for the genotoxicity of *p*-chloro-*o*-toluidine. *p*-Chloro-*o*-toluidine was found to induce DNA damage and gene mutations in *Salmonella typhimurium,* DNA damage in Chinese hamster V79 cells, sister chromatid exchanges (SCE) and chromosomal aberrations in Chinese hamster ovary (CHO) cells, morphological transformation in BALB/c-3T3 cells, DNA binding in calf thymus and mouse and rat liver DNA, and coat color mutations in female C57Bl/6J mice. *p*-Chloro-*o*-toluidine did not induce DNA damage or gene mutations in *Escherichia coli*; SCE in human lymphocytes with or without S9; chromosomal aberrations in CHO cells without S9 activation and human lymphocytes with or without S9; or dominant lethal mutations, heritable translocations, or micronuclei in mice. Unless otherwise specified, rat liver S9 was the source of metabolic activation *in vitro*.

Information presented for studies reviewed by IARC (1990) was often limited to qualitative data with information on study design, doses tested, chemical purity, etc., not provided. In addition, for the sake of simplicity, multiple citations in IARC for the same genetic toxicity assay were discussed as a group rather than individually.

5.1 Noneukaryotic Systems

5.1.1 <u>DNA Damage</u>

Rashid et al. (1984; cited by IARC, 1990) found that *p*-chloro-*o*-toluidine induced DNA damage as measured by differential growth inhibition in repair-proficient and -deficient *S. typhimurium* strains TA1538 and TA1978 [LED = 250 mg/disc (1.7 mmol/disc)], but not in *E. coli* strains WP2, WP2*uvr*A, WP67, CM611, and CM571 [HID = 2000 mg/disc (14.1 mmol/disc)]. Both were tested only in the absence of metabolic activation.

5.1.2 <u>Gene Mutations</u>

IARC (1990) reported conflicting microbial mutagenicity studies with *p*-chloro-*o*-toluidine. In two separate studies it was mutagenic in *S. typhimurium* strain TA1535 in the absence [LED = 163 μg/plate (1.2 μmol/plate)] and strain TA100 in the presence [LED = 7.0 μg/plate (0.05 μmol/plate)] of rat and mouse liver metabolic activation in the standard plate incorporation assay, but less so in the pre-incubation method. In three studies, *p*-chloro-*o*-toluidine was not mutagenic in *S. typhimurium* strains TA1537, TA1538, and TA98 in the presence or absence of rat and hamster liver metabolic activation [HID = 500 (3.5 μmol/plate)]. Most recently, Goggleman et al. (1996) reported that it was mutagenic in *S. typhimurium* strains TA98 [LED = 375 μg/plate (1000 μM)] and TA100 [LED = 100 μg/plate (300 μM)] only in the presence of metabolic activation and negative in strains TA1535 and TA1537 with or without S9.

In Rashid et al. (1984; cited by IARC, 1990), it did not induce *trp* reverse mutations in *E. coli* strains WP2, WP2*uvr*A, WP67, CM611, and CM571 in the presence or absence of metabolic activation [HID = 1000 μg/plate (7.1 μmol/plate)].

5.2 Mammalian Systems *In Vitro*

5.2.1 DNA Damage

Zimmer et al. (1980; cited by IARC, 1990) reported that *p*-chloro-*o*-toluidine induced DNA damage, as tested via alkaline elution, in Chinese hamster V79 cells [LED = 425 µg/mL (3000 µM)]. Galloway et al. (1987; cited by IARC, 1990) found that SCE were induced in CHO cells in the presence and absence of metabolic activation [LED = 50.0 µg/mL (353 µM)]. Bentley et al. (1986) reported that 11,000 µM *p*-[^{14}C]chloro-*o*-toluidine binds to calf thymus DNA after 30 to 60 min in the presence of mouse liver S9 activation. However, most recently, Goggelman et al. (1996) reported that exposure to *p*-chloro-*o*-toluidine at 500 to 2000 µM (-S9) or 250 to 2000 µM (+S9) for one hour did not induce SCE in human peripheral blood lymphocytes in either the presence or absence of metabolic activation.

5.2.2 Chromosomal Damage

Galloway et al. (1987; cited by IARC, 1990) found that *p*-chloro-*o*-toluidine induced chromosomal aberrations in CHO cells only in the presence of metabolic activation [LED = 400 µg/mL (2830 µM)]. However, most recently, Goggelman et al. (1996) reported that exposure to *p*-chloro-*o*-toluidine at 500 to 2000 µM (-S9) or 250 to 2000 µM (+S9) for one hour did not induce chromosomal aberrations in human peripheral blood lymphocytes in either the presence or absence of metabolic activation.

5.2.3 Cell Transformation

Matthews et al. (1993) found that 197 to 842 µM *p*-chloro-*o*-toluidine hydrochloride was active for morphological transformations in BALB/c-3T3 cells clone A31-1-13 in the absence of metabolic activation (LED = 197 µM).

5.3 Mammalian Systems *In Vivo*

5.3.1 DNA Damage

Bentley et al. (1986) reported that *p*-[^{14}C]chloro-*o*-toluidine administered at 25 mg/kg bw (180 µmol/kg) via gastric intubation bound to liver DNA, RNA, and protein in both male MAG mice and Sprague-Dawley rats. DNA binding was greater in the mouse than in the rat. RNA/protein binding was greater in rats than mice.

5.3.2 Gene Mutations

Using the mouse spot test, Lang (1984; cited by IARC, 1990) found that *p*-chloro-*o*-toluidine at an oral dose of 100 mg/kg (706 µmol/kg) induced coat color mutations in female C57Bl/6J mice. It did not, however, induce dominant lethal mutations in mice (strain, doses, and route of administration not provided in IARC, 1990).

5.3.3 Chromosomal Damage

Lang and Adler (1982; cited by IARC, 1990) reported that an oral dose of 200 mg/kg (1410 µmol/kg) *p*-chloro-*o*-toluidine did not induce heritable translocations in SPF NMRI mice. It was also reported that *p*-chloro-*o*-toluidine did not induce micronuclei in mice (strain, doses, and route of administration not provided in IARC, 1990).

Table 5-1. Summary of *p*-Chloro-*o*-toluidine Genotoxicity Studies

Test System	Biological Endpoint	S9 Metab. Activation	Chemical & Purity	Doses Used	Endpoint Response	Comments	Reference
5.1 Noneukaryote Systems							
5.1.1 DNA Damage							
Salmonella typhimurium strains TA1538 and TA1978	DNA damage (growth inhibition)	-	n.p.	n.g.	positive	LED = 250 µg/disc (1.7 mmol/disc)	Rashid et al. (1984; cited by IARC, 1990)
Escherichia coli strains WP2, WP*uvr*A, WP67, CM611, and CM571	DNA damage (growth inhibition)	-	n.p.	n.g.	negative	HID = 2000 µg/disc (14.1 mmol/disc)	Rashid et al. (1984; cited by IARC, 1990)
5.1.2 Gene Mutations							
S. typhimurium strains TA100, TA98, TA1535, TA1537, and TA1538	*his* gene mutations	+/- mouse, rat, or hamster S9	n.p.	n.g.	positive/ positive	Positive in two studies in strain TA100 +S9 (LED = 7.0 µg/plate, 0.05 µmol/plate) and strain TA1535 -S9 (LED = 163.0 µg/plate, 1.2 µmol/plate). All other strains + or -S9 were negative. HID = 500 µg/plate (3.5 µmol/plate).	Three papers cited by IARC (1990)
S. typhimurium strains TA100, TA98, TA1535, and TA1537	*his* gene mutations	+/-	n.p.	100 to 3000 µg/plate (267 to 8000 µM)	positive/ negative	Positive in strain TA100 +S9 [LED = 100 µg/plate (300 µM)] and strain TA98 +S9 [LED = 375 µg/plate (1000 µM)	Goggelman et al. (1996)
E. coli strains WP2, WP2*uvr*A, WP67, CM611, and CM571	*trp* gene mutations	+/-	n.p.	n.g.	negative/ negative	HID = 1000 µg/plate (7.1 µmol/plate)	Rashid et al. (1984; cited by IARC, 1990)
5.2 Mammalian Systems *In Vitro*							
5.2.1 DNA Damage							
calf thymus DNA	DNA binding	+ mouse S9	^{14}C-labeled *p*-chloro-*o*-toluidine	11,000 µM for 30 to 60 min	positive	*p*-[^{14}C]Chloro-*o*-toluidine binds to calf thymus DNA after 30 to 60 min in the presence of mouse liver S9 activation.	Bentley et al. (1986)
Chinese hamster V79 cells	DNA damage (alkaline elution)	-	n.p.	n.g.	positive	LED = 425 µg/mL (3000 µM)	Zimmer et al. (1980; cited by IARC, 1990)
Chinese hamster ovary (CHO) cells	sister chromatid exchanges (SCE)	+/-	n.p.	n.g.	positive/ positive	LED = 50.0 µg/mL (350 µM)	Galloway et al. (1987; cited by IARC, 1990)

Table 5-1. Summary of *p*-Chloro-*o*-toluidine Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Chemical & Purity	Doses Used	Endpoint Response	Comments	Reference
human peripheral blood lymphocytes	SCE	+/-	n.p.	500 to 2000 μM (-S9), 250 to 2000 μM (+S9) for 1 h	negative/ negative	25 metaphases were scored per culture	Goggelman et al. (1996)
5.2.2 Chromosomal Damage							
CHO cells	chromosomal aberrations	+/-	n.p.	n.g.	positive/ negative	+S9 LED = 400 μg/mL (2830 μM), -S9 HID not provided.	Galloway et al. (1987; cited by IARC, 1990)
human peripheral blood lymphocytes	chromosomal aberrations	+/-	n.p.	500 to 2000 μM (-S9), 250 to 2000 μM (+S9) for 1 h	negative/ negative	100 metaphases were scored per culture	Goggelman et al. (1996)
5.2.3 Cell Transformation							
BALB/c-3T3 cells clone A31-1-13	morphological transformation	NA	n.p.	197 to 842 μM for 48 h	positive	LED = 197 μM	Matthews et al. (1993)
5.3 Mammalian Systems *In Vivo*							
5.3.1 DNA Damage							
male MAG mice	DNA/RNA/protein binding	NA	^{14}C-labeled *p*-chloro-*o*-toluidine	25 mg/kg (180 μmol/kg) via gastric intubation	positive	DNA binding was greater than RNA or protein binding.	Bentley et al. (1986)
male Sprague Dawley rats	DNA/RNA/protein binding	NA	^{14}C-labeled *p*-chloro-*o*-toluidine	25 mg/kg (180 μmol/kg) via gastric intubation	positive	RNA and protein binding was greater than DNA binding.	Bentley et al. (1986)
5.3.2 Gene Mutations							
female C57Bl/6J mice	coat color mutations (mouse spot test)	NA	n.p.	100 mg/kg (706 μmol/kg) orally	positive		Lang (1984; cited by IARC, 1990)
mice (strain not provided)	dominant lethal mutations	NA	n.p.	n.g.	negative	Doses and route of exposure were not given.	IARC (1990); original paper not cited

Table 5-1. Summary of *p*-Chloro-*o*-toluidine Genotoxicity Studies (Continued)

Test System	Biological Endpoint	S9 Metab. Activation	Chemical & Purity	Doses Used	Endpoint Response	Comments	Reference
5.3.3 Chromosomal Damage							
SPF NMRI mice	heritable translocations	NA	n.p.	200 mg/kg (1410 µmol/ kg) orally	negative		Lang and Adler (1982; cited by IARC, 1990)
mice (strain not provided)	micronuclei formation	NA	n.p.	n.g.	negative	Doses and route of exposure were not given.	IARC (1990); original paper not cited

Abbreviations: HID = highest ineffective dose; LED = lowest effective dose; NA = not applicable; n.g. = not given; n.p. = not provided

Figure 5-1. Genetic Activity of Profile of *p*-Chloro-*o*-toluidine (Data limited to IARC, 1990)

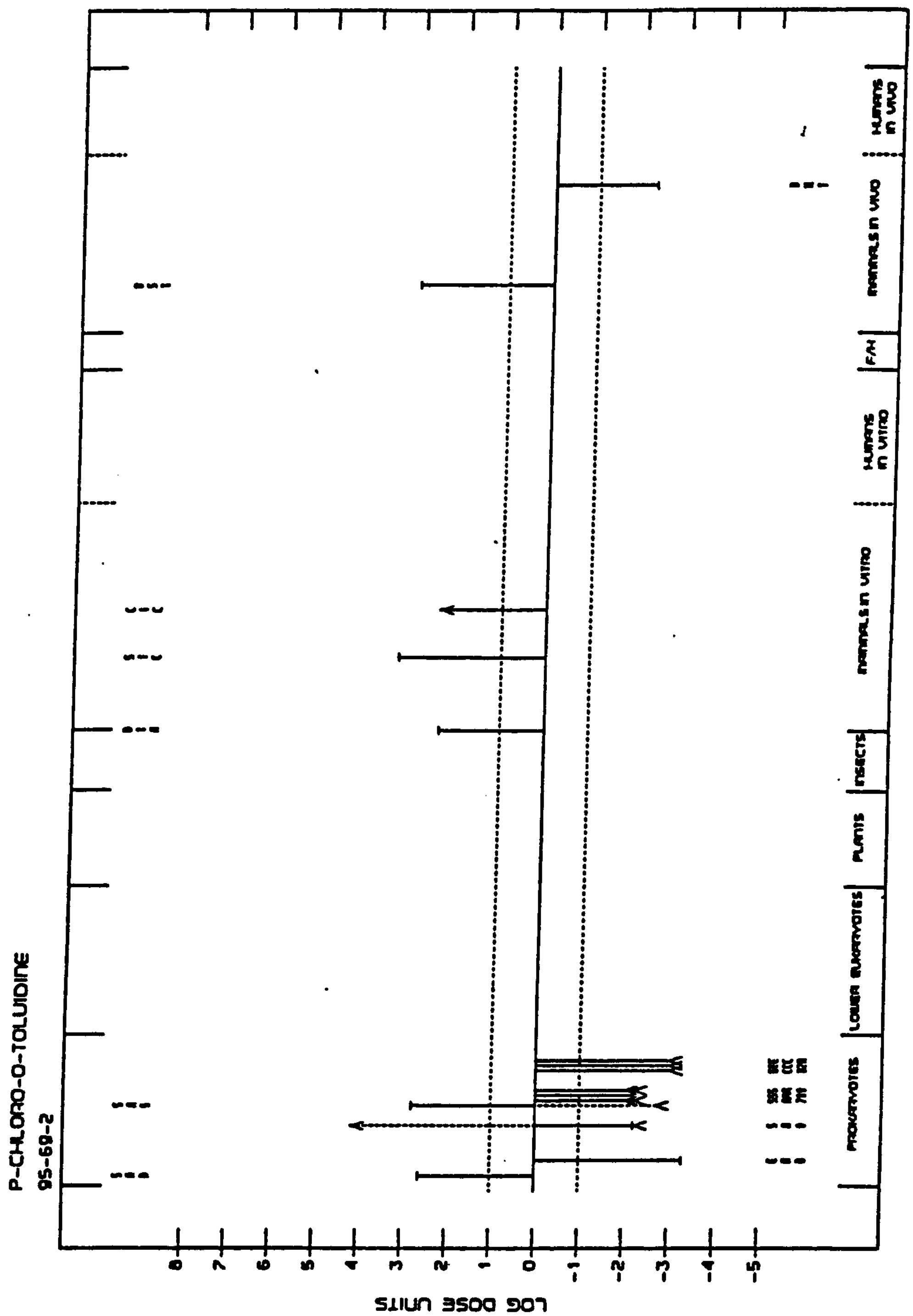
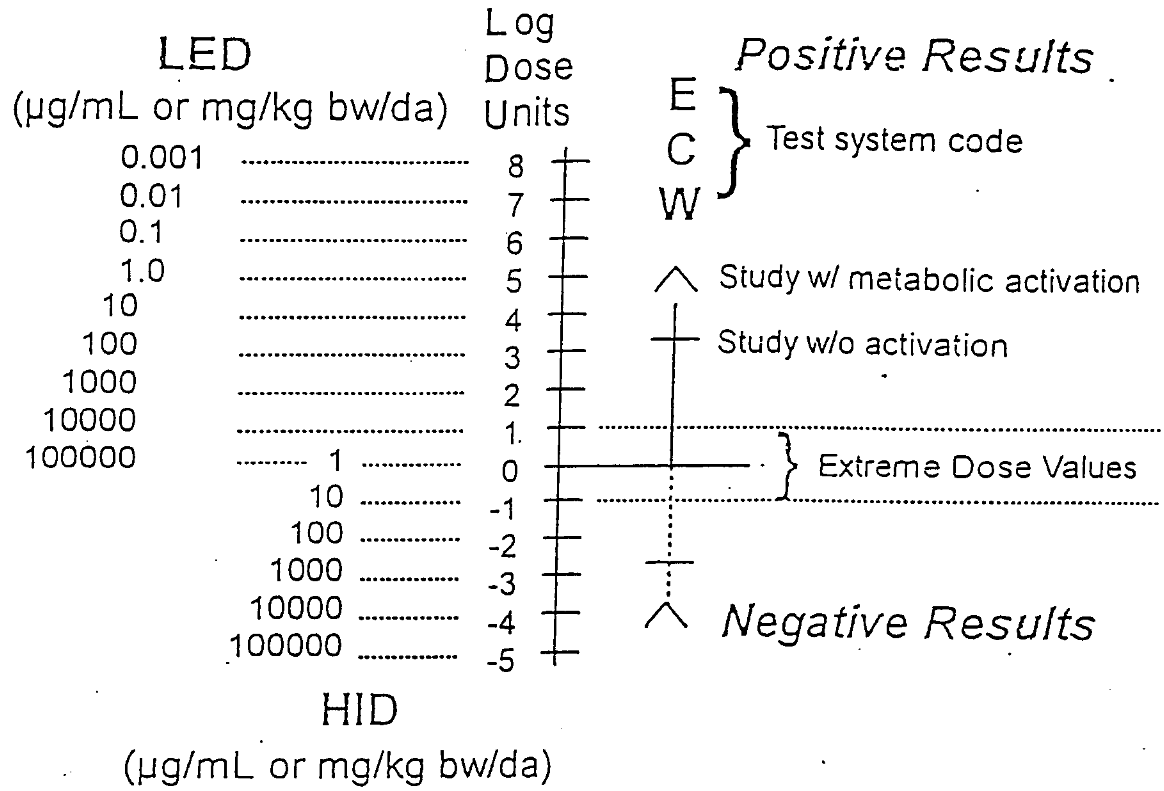


Figure 5-2. Schematic View of a Genetic Activity Profile



A schematic view of a Genetic Activity Profile (GAP) representing four studies (two positive and two negative) for an example short-term test, ECW. Either the lowest effective dose (LED) or the highest ineffective dose (HID) is recorded from each study, and a simple mathematical transformation (as illustrated above) is used to convert LED or HID values into the logarithmic dose unit (LDU) values plotted in a GAP. For each test, the average of the LDUs of the majority call is plotted using a solid vertical bar drawn from the origin. A dashed vertical bar indicates studies that conflict with the majority call for the test. Note in cases where there are an equal number of positive and negative studies, as shown here, the overall call is determined positive. The GAP methodology and database have been reported previously (Garrett et al., 1984; Waters et al., 1988, 1991).

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6.0 OTHER RELEVANT DATA

Summary: Within 72 hours of administration of *p*-chloro-*o*-toluidine hydrochloride, 71% of the dose was eliminated in the urine and ~25% in feces of male and female rats. Metabolite patterns in the urine of mice and rats indicated that *p*-chloro-*o*-toluidine hydrochloride was metabolized differently in the two species. In incubations containing phenobarbital (PB)-induced liver microsomes exposed to [^{14}C-methyl]*p*-chloro-*o*-toluidine, the reactive metabolite 5-chloro-2-hydroxylaminotoluene was produced. [^{14}C-Methyl]*p*-chloro-*o*-toluidine incubated with an unspecified liver microsomal reaction system containing NADPH for 30 min, produced a major metabolite, 4-chloro-2-methylphenylhydroxylamine (CMPHA) and a minor metabolite, 4,4′-dichloro-2,2′-dimethylazobenzene.

6.1 Metabolism, Absorption, Distribution, and Excretion

Following oral administration of [^{14}C-methyl]*p*-chloro-*o*-toluidine to male and female albino rats, 71% of the administered radioactivity was eliminated in the urine and 24.5% was detected in the feces within 72 hours (Knowles and Gupta, 1970; cited by IARC, 1990).

The metabolism of ^{14}C-labeled *p*-chloro-*o*-toluidine and *p*-chloro-*o*-toluidine hydrochloride has been studied in male and female rats as well as in male mice. After male (Tif: MAG f [SPF]) mice and male Sprague-Dawley rats were orally administered a single dose of 25 mg/kg [140 µmol/kg] bw [^{14}C]*p*-chloro-*o*-toluidine hydrochloride, preliminary analysis of metabolite patterns in the urine of these animals indicated that *p*-chloro-*o*-toluidine hydrochloride was metabolized differently in the two species (Bentley et al., 1986; see Section 6.3.1.1).

Hill et al. (1979; cited by IARC, 1990) reported that the reactive metabolite 5-chloro-2-hydroxylaminotoluene was produced in incubations containing phenobarbital (PB)-induced liver microsomes exposed to [^{14}C-methyl]*p*-chloro-*o*-toluidine.

Other studies related to *p*-chloro-*o*-toluidine *in vitro* metabolism were conducted by Struck et al. (1978), who incubated [^{14}C-methyl]*p*-chloro-*o*-toluidine with an unspecified liver microsomal reaction system containing NADPH for 30 min. After chloroform extraction of the reaction mixture, the extract was fractionated by silica gel thin-layer chromatography, yielding a single band. Using mass spectral (MS) and thin-layer chromatography (TLC) comparisons with authentic standards, the authors identified the major metabolite, calling it CMPHA. CMPHA, apparently the same compound identified by Hill et al., was also identified as the major metabolite produced in an identical microsomal fraction, but separated by paper chromatography in 0.3 M NaCl. A minor metabolite, 4,4′-dichloro-2,2′-dimethylazobenzene, was also separated by paper chromatography. Identified by MS and TLC comparison with an authentic standard, the structure was confirmed by proton magnetic resonance spectroscopy.

6.2 Pharmacokinetics

No data were available.

6.3 Modes of Action (Metabolism and Genotoxicity)

Summary: *p*-Chloro-*o*-toluidine is clearly positive in genotoxicity assays (FAO/WHO, 1985; cited by Wu et al., 1989; see also Section 5.0). Radioactivity from [^{14}C-methyl]*p*-chloro-*o*-toluidine hydrochloride i.p. administered to rats was found bound to liver DNA, RNA, and protein. Binding to hepatic DNA in mice was approximately twofold higher in mice than in rats at 6, 12, and 20 hours post-dose following oral administration of [^{14}C-methyl]*p*-chloro-*o*-toluidine hydrochloride; at all time points, the extent of binding decreased in the order: mice, DNA > RNA or protein binding; rats, RNA and protein > DNA binding.

Mouse liver fractions catalyzed the binding of *p*-chloro-*o*-toluidine hydrochloride to calf thymus DNA more readily than rat liver S9 fractions. Conversely, binding to protein and RNA was more marked in the presence of rat S9 than in incubations containing mouse S9. "The extent of binding to DNA, but not that of binding to RNA or protein, correlated with the known differences in the susceptibility of rats and mice to the tumorigenicity of the compound" (Bentley et al., 1986).

Following a 3-day exposure of *p*-chloro-*o*-toluidine to rats, no observable effects on splenic tumoricidal effector cell functions were found, suggesting that *p*-chloro-*o*-toluidine does elicit its carcinogenic effects by impairing the immune function in rats (Thomas et al., 1990).

6.3.1 <u>Adduct formation</u>

Hill et al. (1979; cited by IARC, 1990) i.p. administered 14 mg/kg bw [^{14}C-methyl]*p*-chloro-*o*-toluidine hydrochloride to Osborne-Mendel rats and found radioactivity bound to liver DNA, RNA, and protein. In other tissues, these macromolecules contained little radioactivity.

These findings have been confirmed by Bentley et al. (1986) after [^{14}C]*p*-chloro-*o*-toluidine hydrochloride (25 mg/kg; [140 µmol/kg]) was orally administered to male (TIF: MAG f [SPF]) mice and male Sprague-Dawley rats. These results showed that binding to hepatic DNA in mice was approximately twofold higher than in rats at 6, 12, and 20 hours post-dose. These data also showed that at all time points, the extent of binding decreased in the order: mice, DNA > RNA or protein binding; rats, RNA and protein > DNA binding (see Table 5-1).

In vitro studies of incubations containing 4.8 mg calf thymus DNA, 1.3 mg NADP, and mouse or rat liver S9 fractions (3 to 8 mg protein) showed that mouse liver fractions catalyzed the binding of *p*-chloro-*o*-toluidine to calf thymus DNA more readily than rat liver S9 fractions. Conversely, binding to protein and RNA was more marked in the presence of rat S9 than in the incubations containing mouse S9 (Bentley et al., 1986). However, no observation of species differences in DNA repair rates was noted, and these results failed to demonstrate a preferential persistence of binding to mouse liver non-parenchymal cell DNA.

Two major DNA adducts (unidentified) were formed *in vitro* in incubations containing mouse liver or rat liver fractions and *p*-chloro-*o*-toluidine hydrochloride, but one of these adducts was formed to a much greater extent (6- to 30-fold) in mouse incubations than in rat. These findings suggest that different patterns of reactive metabolites may be formed from *p*-chloro-*o*-toluidine in mice and rats, with mice producing metabolites with a preference for DNA binding, and rats producing metabolites that display a higher affinity for proteins. Bentley et al. (1986), who found a higher DNA binding in mouse liver macromolecules (8 pmol/mg) vs. rats (4.8 pmol/mg), did not detect notable DNA damage in the target tissues—capillary endothelial cells. However, the author noted that the "extent of binding to DNA, but not that of binding to RNA or protein, correlated with the known differences in the susceptibility of rats and mice to the tumorigenicity of the compound" (Bentley et al., 1986).

6.3.2 Role of Tumoricidal Effector Cells and Carcinogenicity

The capacity of a chemical to impair immune function may be related to its carcinogenic potential (Davidson et al., 1956; cited by Thomas et al., 1990), particularly those chemicals that influence tumoricidal effector cell populations, such as the natural killer (NK) and natural cytotoxic (NC) cell populations. These cells have the ability to lyse certain tumor cell targets without prior sensitization (Kiessling and Haller, 1978; cited by Thomas et al., 1990). Large granular lymphocytes that spontaneously lyse lymphoma targets during a 4-hour period may be termed NK activity, and lymphocytes that preferentially lyse solid tumor targets over 16 hours are natural cytotoxic cells (Ortaldo and Reynolds, 1978, and Stutman et al., 1978; cited by Thomas et al., 1990).

Thomas et al. (1990) studied the immunotoxic effects of *p*-chloro-*o*-toluidine on spleen-derived lymphoid cell populations from Sprague-Dawley rats following a 3-day exposure to 0, 10, 50, or 100 mg/kg *p*-chloro-*o*-toluidine [56, 280, or 560 μmol/kg], and found no observable effects on splenic tumoricidal effector cell functions (NK and NC activity). In addition, "mitogenic response of splenocytes to concanavalin (Con-A) and lipopolysaccharide (LPS), which are indicators of T-cell-mediated immunity and humoral immunity, respectively, did not exhibit any change with either treatment" (Thomas et al., 1990). The results indicated that p-chloro-o-toluidine carcinogenicity in rats is not related to its capacity to impair immune function by altering tumoricidal effector cell populations.

6.4 Structure-Activity Relationships

The amino group of *p*-chloro-*o*-toluidine was identified as an alerting substructure responsible for binding with DNA. Computer Automated Structure Evaluation (CASE) was used to classify *p*-chloro-*o*-toluidine hydrochloride as a cryptic mutagen. CASE identified two biophobes/biophores that are present in *p*-chloro-*o*-toluidine and its hydrochloride that have potential for mutagenicity in *S. typhimurium*: Cl-CH= and NH_2-C=CH-CH=C-CH-.

p-Chloro-*o*-toluidine is structurally related to two other rodent carcinogens: *o*-toluidine hydrochloride and chlordimeform. *o*-Toluidine hydrochloride administered in the diet to female and male B6C3F$_1$ mice increased the incidences of hepatocellular carcinomas and adenomas in females and hemangiosarcomas at multiple sites in males, and hemangiosarcomas and hemangiomas of the abdominal viscera in male and female mice. Hemangiomas and hemangiosarcomas were found in male mice administered chlordimeform in the diet.

6.4.1 Identification of Structural Alerts

Ashby and Tennant (1988) conducted a survey of 222 chemicals (including *p*-chloro-*o*-toluidine hydrochloride) that had been evaluated for carcinogenicity in mice and rats by the United States NCI/NTP. Potential electrophilic reactive sites were identified for each of the chemicals evaluated. The authors identified the amino group as an alerting substructure responsible for binding with DNA.

Subsequently, Rosenkranz and Klopman (1990a) used CASE, to classify *p*-chloro-*o*-toluidine hydrochloride as a "cryptic mutagen." Cryptic mutagens were defined as agents that possess structural alerts for potential mutagenicity in *S. typhimurium* (Ashby and Tennant, 1988; Ashby et al., 1989) without this potential being expressed in *S. typhimurium*, although the agents are carcinogenic in rodents and humans (Rosenkranz and Klopman, 1990a). CASE has identified 41 significant biophores and biophobes associated with *S. typhimurium* mutagenicity. All of these parameters were used to calculate the likelihood that a chemical is a mutagen. Of the 41 structural descriptors, two are present in *p*-chloro-*o*-toluidine and its hydrochloride: Cl-CH= (P = 0.031, active, +++ [increasing activity from + to +++]) and NH_2-C =CH-CH=C-CH- (P = 0.016; active, +++).

6.4.2 Structurally Related Carcinogens

6.4.2.1 *o*-Toluidine Hydrochloride

o-Toluidine hydrochloride administered in the diet to female and male B6C3F_1 mice increased the incidences of hepatocellular carcinomas and adenomas in females and hemangiosarcomas at multiple sites in males, and hemangiosarcomas and hemangiomas of the abdominal viscera in both sexes of CD-1 mice (IARC V. 16, 1978; IARC V.27, 1982; IARC S.4, 1982; IARC S.7, 1987; all cited by NTP 44, 1996).

6.4.2.2 Chlordimeform

p-Chloro-*o*-toluidine is a metabolite of the pesticide chlordimeform. Hemangiomas and hemangiosarcomas were found in male mice administered chlordimeform in the diet. A dose-related response was seen in the low-, mid-, and high-dose groups (Li, 1985 [from an abstract of the Chinese publication]).

Leslie et al. (1988) reported that administration of *p*-chloro-*o*-toluidine to male rats was associated with changes in the hepatic xenobiotic biotransformation system as demonstrated with the use of microsomal assays and sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The authors reported induction of ethoxyresorufin-*O*-deethylase (EROD) activity in rats at a dose of 10 mg/kg by *p*-chloro-*o*-toluidine or *o*-toluidine, whereas a dose of 50 mg/kg chlordimeform (precursor to *p*-chloro-*o*-toluidine) was required before induction of EROD activity. The authors stated that "this is consistent with lower doses of *p*-chloro-*o*-toluidine than chordimeform being required to produce carcinomas" in mice as seen in studies conducted by FAO (1979/1980; cited by Leslie et al., 1988). Treatment of rats with *p*-chloro-*o*-toluidine resulted in increased EROD activity only; in contrast to the study by Creaven and Parke (1966; cited by Leslie et al., 1988) and Parke (1983; cited by Leslie et al., 1988), who found that concurrent decrease in aldrin epoxidation and elevation of EROD activity are characteristic of many carcinogens. However, the induction of EROD activity alone is sufficient to raise some suspicion regarding the carcinogenicity of a chemical (Ioannides et al., 1984; cited by Leslie et al., 1988). [Thus, concurrent elevation of EROD activity and carcinogenic response at doses one-fifth those necessary to elicit similar responses following chlordimeform treatment suggests a mode of action that involves induction of EROD activity.]

6.5 Cell Proliferation

Experimental details for the studies described in this section are presented in Table 6-1.

Summary: There was no significant increase in the incorporation of [^{3}H]thymidine into subcutaneous capillary endothelial cells of specific-pathogen-free male mice (strain not specified) and specific-pathogen-free male Sprague Dawley rats following oral administration of a single dose or of 14-daily doses of *p*-chloro-*o*-toluidine (Bentley et al., 1986).

Ciba-Geigy (1974a, 1974b) reported that administration of 20, 100, or 500 ppm *p*-chloro-*o*-toluidine in the diet of male and female ICR mice and Sprague-Dawley rats for 80 weeks mainly affected the kidneys: Male and female ICR mice had an increased incidence of focal inflammation and male Sprague-Dawley rats had an increased incidence of chronic nephritis. In female rats, the incidence of chronic nephritis was increased, as compared to controls, in the low-dose group, but not in the mid- and high-dose groups. No studies were found that evaluated whether *p*-chloro-*o*-toluidine hydrochloride induced cell proliferation in experimental animals.

6.5.1 <u>*p*-Chloro-*o*-toluidine</u>

6.5.1.1 Mice

There was no significant increase in the incorporation of [^{3}H]thymidine into subcutaneous capillary endothelial cells of specific-pathogen-free male mice following oral administration of a single dose or of 14-daily doses of 25 mg *p*-chloro-*o*-toluidine per kilogram mean body weight (180 μmol/kg bw) (Bentley et al., 1986).

There was a dose-dependent increase in the incidence of focal inflammation of the kidneys (evaluated histologically) in male and female ICR mice administered 20, 100, or 500 ppm *p*-chloro-*o*-toluidine in the diet for 80 weeks (13/19 low-, 6/9 mid-, and 1/1 high-dose males vs. 9/21 controls; 6/11 low- and 5/5 mid-dose females vs. 7/16 controls; no high-dose females and only 1 high-dose male survived the treatment period). Significant cell proliferation was not detected in a number of other tissues (Ciba-Geigy, 1974a).

6.5.1.2 Rats

There was no significant increase in the incorporation of [^{3}H]thymidine into subcutaneous capillary endothelial cells of specific-pathogen-free male Sprague-Dawley rats following oral administration of a single dose or of 14-daily doses of 25 mg *p*-chloro-*o*-toluidine per kilogram mean body weight (180 μmol/kg bw) (Bentley et al., 1986).

There was an increase in the incidence of chronic nephritis in male Sprague-Dawley rats administered 20, 100, or 500 ppm *p*-chloro-*o*-toluidine in the diet for 80 weeks (10/10 low-, 5/6 mid-, and 9/9 high-dose males vs. 9/13 controls). In females, the incidence of chronic nephritis was increased, as compared to controls, in the low-dose group, but not in the mid- or high-dose groups. Inflammation of fatty tissue was detected in 1/9 high-dose males, but not in any other rats. Significant cell proliferation was not detected in a number of other tissues (Ciba-Geigy, 1974b).

6.5.2 <u>*p*-Chloro-*o*-toluidine hydrochloride</u>

No studies were found that evaluated whether *p*-chloro-*o*-toluidine hydrochloride induced cell proliferation in experimental animals.

Table 6-1. Cell Proliferation Induced by *p*-Chloro-*o*-toluidine

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Mice							
specific-pathogen-free mice (age and strain not specified)	3M	3M (H_2O alone)	*p*-chloro-*o*-toluidine, >99% pure	25 mg/kg bw (180 µmol/kg bw), orally administered	single oral dose	Oral administration of *p*-chloro-*o*-toluidine was followed 6, 9, 12, and 15 hours later by i.p. injection of [^{3}H]thymidine (500 µCi/kg bw per application). Mice were killed 3 hours after the last i.p. injection (18 hours after initial treatment with *p*-chloro-*o*-toluidine).	Bentley et al. (1986)
	3M	3M (H_2O alone)	*p*-chloro-*o*-toluidine, >99% pure	25 mg/kg bw (180 µmol/kg bw), orally administered	single oral dose	Oral administration of *p*-chloro-*o*-toluidine was followed 25, 28, 31, and 34 hours later by i.p. injection of [^{3}H]thymidine (500 µCi/kg bw per application). Mice were killed 3 hours after the last i.p. injection (37 hours after initial treatment with *p*-chloro-*o*-toluidine).	
	3M	2M (H_2O alone)	*p*-chloro-*o*-toluidine, >99% pure	25 mg/kg bw/day (180 µmol/kg bw), orally administered	14 days	The fourteenth dose of *p*-chloro-*o*-toluidine was followed 6, 9, 12, and 15 hours later by i.p. injection of [^{3}H]thymidine (500 µCi/kg bw per application). Mice were killed 3 hours after the last i.p. injection (18 hours after administration of the last *p*-chloro-*o*-toluidine dose). **Capillary Endothelial Cells:** Negative After sacrifice, blood capillary endothelial cells were isolated from the dorsal skin of the mice. None of the 3 treatment schedules resulted in significant increases in the labeling index (measured as the total counted nuclei per mouse that contained 5-12 silver grains) of the capillary endothelial cells.	
?-wk-old ICR mice	30M, 30F per dose	30M, 30F (untreated)	*p*-chloro-*o*-toluidine, purity not specified	20, 100, or 500 ppm in diet	80 wk	Surviving mice were killed at the end of the treatment period. All HD females died by 67 weeks. Only 1 HD male survived at 80 weeks. The following tissues were examined: heart, lungs, spleen, liver, kidneys, stomach, small intestine, testes, ovaries, adrenal glands, pancreas, eyes, pituitary gland, thyroid gland, thymus gland, lymph nodes, and urinary bladder. *Sacrificed Mice*: **Kidney:** There was an increase in the incidence of focal inflammation in treated mice as compared to controls (13/19 LD, 6/9 MD, and 1/1 HD males vs. 9/21 controls; 6/11 LD and 5/5 MD females vs. 7/16 controls). Significant renal tumors were not detected in treated mice. **Other:** Significant cell proliferation was not detected in other tissues.	Ciba-Geigy (1974a)

Table 6-1. Cell Proliferation Induced by *p*-Chloro-*o*-toluidine (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
Rats							
specific-pathogen-free Sprague-Dawley rats (age not specified)	3M	3M	*p*-chloro-*o*-toluidine, >99% pure	25 mg/kg bw (180 µmol/kg bw), orally administered	single oral dose	Oral administration of *p*-chloro-*o*-toluidine was followed 6, 9, 12, and 15 hours later by i.p. injection of [^{3}H]thymidine (500 µCi/kg bw per application). Rats were killed 3 hours after the last i.p. injection (18 hours after initial treatment with *p*-chloro-*o*-toluidine).	Bentley et al. (1986)
	3M	3M	*p*-chloro-*o*-toluidine, >99% pure	25 mg/kg bw (180 µmol/kg bw), orally administered	single oral dose	Oral administration of *p*-chloro-*o*-toluidine was followed 25, 28, 31, and 34 hours later by i.p. injection of [^{3}H]thymidine (500 µCi/kg bw per application). Rats were killed 3 hours after the last i.p. injection (37 hours after initial treatment with *p*-chloro-*o*-toluidine).	
	3M	2M	*p*-chloro-*o*-toluidine, >99% pure	25 mg/kg bw/day (180 µmol/kg bw), orally administered	14 days	The fourteenth dose of *p*-chloro-*o*-toluidine was followed 6, 9, 12, and 15 hours later by i.p. injection of [^{3}H]thymidine (500 µCi/kg bw per application). Rats were killed 3 hours after the last i.p. injection (18 hours after administration of the last *p*-chloro-*o*-toluidine dose). **Capillary Endothelial Cells:** Negative After sacrifice, blood capillary endothelial cells were isolated from the dorsal skin of the rats. None of the 3 treatment schedules resulted in significant increases in the labeling index (measured as the total counted nuclei per mouse that contained 5-12 silver grains) of the capillary endothelial cells.	

Table 6-1. Cell Proliferation Induced by *p*-Chloro-*o*-toluidine (Continued)

Age, Strain, Species	No./Sex Exposed	Controls	Chemical Form and Purity	Dose	Duration of Exposure	Results/Comments	Reference
?-wk-old Sprague-Dawley rats	30M, 30F per dose	30M, 30F	*p*-chloro-*o*-toluidine, purity not specified	20, 100, or 500 ppm in diet	*males*: 94 wk *females*: 104 wk	Surviving rats were killed at the end of the treatment period. The following tissues were examined: heart, lungs, spleen, liver, kidneys, stomach, small intestine, testes, ovaries, adrenal glands, pancreas, eyes, pituitary gland, thyroid gland, thymus gland, lymph nodes, and urinary bladder. **Kidney**: There was an increase in the incidence of chronic nephritis in males as treated compared to controls (10/10 LD, 5/6 MD, and 9/9 HD males vs. 9/13 controls). In females, the incidence of chronic nephritis was increased as compared to controls with the LD, but not with the MD or HD. Papillary adenocarcinoma was detected in 1/6 HD females, but not in any other rats. **Abdominal Cavity:** Inflammation of fatty tissue was detected in 1/9 HD males, but not in any other rats. No significant abdominal cavity tumors were detected in treated rats. **Other:** Significant cell proliferation was not detected in other tissues.	Ciba-Geigy (1974b)

Abbreviations: bw = body weight; F = females; HD = high dose; i.p. = intraperitoneal; LD = low dose; M = males; MD = mid dose

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APPENDIX A

DESCRIPTION OF ONLINE SEARCHES FOR *p*-CHLORO-*o*-TOLUIDINE AND *p*-CHLORO-*o*-TOLUIDINE HYDROCHLORIDE

DESCRIPTION OF ONLINE SEARCHES FOR *p*-CHLORO-*o*-TOLUIDINE AND *p*-CHLORO-*o*-TOLUIDINE HYDROCHLORIDE (IARC Monograph in Vol. 48, 1990)

The searches described below were conducted between January and October 1996. An exhaustive search of all pertinent databases was not attempted, but the ones chosen were expected to provide citations for most of the relevant recently published literature. No attempt was made in the search strategy to find toxicity information for metabolites and other structural analogs.

Generally, if an IARC monograph or another authoritative review had been published, literature searches were generally restricted from the year before publication to the current year.

Older literature that needed to be examined was identified from the reviews and original articles as they were acquired. Current awareness was maintained by conducting weekly searches of Current Contents on Diskette® Life Sciences 1200 [journals] edition.

<u>TOXLINE</u> (on STN International): Use of the Chemical Abstracts Service Registry Numbers (CASRNs) for the two compounds found 119 records in the entire database (1940s to January 1996). About 15% were duplicates, 21% had been identified by other searches (primarily EMIC and EMICBACK), and 39% (46) appeared to be of interest to judge merely from their titles. Dr. H.B. Matthews directed the selection process.

<u>CANCERLIT</u>: Only 10 records were indexed by the CASRNs (only that of the free base) in the entire database (1963 to 1996). These 10 publications had already been identified in other database searches.

<u>EMBASE</u>: One of the 5 records retrieved by using the CASRNs of the 2 compounds was unique to this database.

<u>EMIC/EMICBACK</u>: Four records were indexed in EMIC by the CASRNs of the 2 compounds; the one reference with original data was acquired. EMICBACK had 21 records indexed by the CASRNs.

<u>IRIS</u>: Neither of the 2 compounds was listed.

<u>MEDLINE</u>: In the entire database (1966 to 1996), 14 records were indexed by the CASRNs of the 2 compounds (only that of the free base). Before completion of checks for duplication with other databases, about 6 records were tentatively selected for acquisition of the publications.

<u>TOXLIT</u>: In the entire database (1965 to 06 March 1996), 135 records were indexed by the CASRNs; of these, 52 had been published since 1988. These records were further reduced to 34 by combining with the truncated (use of ? with the word stem) free text terms in the statement "carcinogen? or mechanism? or toxicokinetic? or pharmacokinetic? or metaboli? or neoplas? or

hyperplas? or metaplas? or foci? or tumor? or tumour?". Before complete checks for duplication with other databases, about 20 publications were selected for acquisition.

TSCATS (Toxic Substances Control Act Test Submissions): All 9 of the studies in the database were acquired.

In September 1996, the contractor performed searches for updating sections 1 and 2, which had been last updated in 1994 with regulatory information from print sources and REGMAT (May 1993 version). REGMAT had broad coverage of EPA regulations, but it is no longer available. Databases searched in 1996 included CSCHEM and CSCORP for U.S. suppliers (databases produced by Chem Sources); HSDB; the Chemical Information System's databases SANSS (the Structure and Nomenclature Search System) and ISHOW (for physical-chemical properties); Chemical Abstracts Service's (CAS) File CHEMLIST for TSCA and SARA updates in 1996; and CAS's CA File sections 59 (Air Pollution and Industrial Hygiene), 60 (Waste Disposal and Treatment), and 61 (Water) for environmental exposure information.

APPENDIX B

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

LISTING OF GAP TEST CODES IN ALPHABETICAL ORDER

Test Code	Definition
ACC	Allium cepa, chromosomal aberrations
AIA	Aneuploidy, animal cells in vitro
AIH	Aneuploidy, human cells in vitro
ANF	Aspergillus nidulans, forward mutation
ANG	Aspergillus nidulans, genetic crossing-over
ANN	Aspergillus nidulans, aneuploidy
ANR	Aspergillus nidulans, reverse mutation
ASM	Arabidopsis species, mutation
AVA	Aneuploidy, animal cells in vivo
AVH	Aneuploidy, human cells in vivo
BFA	Body fluids from animals, microbial mutagenicity
BFH	Body fluids from humans, microbial mutagenicity
BHD	Binding (covalent) to DNA, human cells in vivo
BHP	Binding (covalent) to RNA or protein, human cells in vivo
BID	Binding (covalent) to DNA in vitro
BIP	Binding (covalent) to RNA or protein in vitro
BPF	Bacteriophage, forward mutation
BPR	Bacteriophage, reverse mutation
BRD	Other DNA repair-deficient bacteria, differential toxicity
BSD	Bacillus subtilis rec strains, differential toxicity
BSM	Bacillus subtilis multi-gene test
BVD	Binding (covalent) to DNA, animal cells in vivo
BVP	Binding (covalent) to RNA or protein, animal cells in vivo
CBA	Chromosomal aberrations, animal bone-marrow cells in vivo
CBH	Chromosomal aberrations, human bone-marrow cells in vivo
CCC	Chromosomal aberrations, spermatocytes treated in vivo and cytes obs.
CGC	Chromosomal aberrations, spermatogonia treated in vivo and cytes obs.
CGG	Chromosomal aberrations, spermatogonia treated in vivo and gonia obs.
CHF	Chromosomal aberrations, human fibroblasts in vitro
CHL	Chromosomal aberrations, human lymphocyte in vitro
CHT	Chromosomal aberrations, transformed human cells in vitro
CIA	Chromosomal aberrations, other animal cells in vitro
CIC	Chromosomal aberrations, Chinese hamster cells in vitro
CIH	Chromosomal aberrations, other human cells in vitro
CIM	Chromosomal aberrations, mouse cells in vitro
CIR	Chromosomal aberrations, rat cells in vitro
CIS	Chromosomal aberrations, Syrian hamster cells in vitro
CIT	Chromosomal aberrations, transformed animal cells in vitro
CLA	Chromosomal aberrations, animal leukocytes in vivo
CLH	Chromosomal aberrations, human lymphocytes in vivo

Test Code	Definition
COE	Chromosomal aberrations, oocytes or embryos treated in vivo
CVA	Chromosomal aberrations, other animal cells in vivo
CVH	Chromosomal aberrations, other human cells in vivo
DIA	DNA strand breaks, cross-links or rel. damage, animal cells in vitro
DIH	DNA strand breaks, cross-links or rel. damage, human cells in vitro
DLM	Dominant lethal test, mice
DLR	Dominant lethal test, rats
DMC	Drosophila melanogaster, chromosomal aberrations
DMG	Drosophila melanogaster, genetic crossing-over or recombination
DMH	Drosophila melanogaster, heritable translocation test
DML	Drosophila melanogaster, dominant lethal test
DMM	Drosophila melanogaster, somatic mutation (and recombination)
DMN	Drosophila melanogaster, aneuploidy
DMX	Drosophila melanogaster, sex-linked recessive lethal mutation
DVA	DNA strand breaks, cross-links or rel. damage, animal cells in vivo
DVH	DNA strand breaks, cross-links or rel. damage, human cells in vivo
ECB	Escherichia coli (or E. coli DNA), strand breaks, cross-links or repair
ECD	Escherichia coli pol A/W3110-P3478, diff. toxicity (spot test)
ECF	Escherichia coli (excluding strain K12), forward mutation
ECK	Escherichia coli K12, forward or reverse mutation
ECL	Escherichia coli pol A/W3110-P3478, diff. toxicity (liquid susp. test)
ECR	Escherichia coli, miscellaneous strains, reverse mutation
ECW	Escherichia coli WP2 uvrA, reverse mutation
EC2	Escherichia coli WP2, reverse mutation
ERD	Escherichia coli rec strains, differential toxicity
FSC	Fish, chromosomal aberrations
FSI	Fish, micronuclei
FSM	Fish, mutation
FSS	Fish, sister chromatid exchange
FSU	Fish, unscheduled DNA synthesis
GCL	Gene mutation, Chinese hamster lung cells exclusive of V79 in vitro
GCO	Gene mutation, Chinese hamster ovary cells in vitro
GHT	Gene mutation, transformed human cells in vivo
GIA	Gene mutation, other animal cells in vitro
GIH	Gene mutation, human cells in vitro
GML	Gene mutation, mouse lymphoma cells exclusive of L5178Y in vitro
GVA	Gene mutation, animal cells in vivo
G5T	Gene mutation, mouse lymphoma L5178Y cells in vitro, TK locus
G51	Gene mutation, mouse lymphoma L5178Y cells in vitro, all other loci
G9H	Gene mutation, Chinese hamster lung V-79 cells in vitro, HPRT locus
G9O	Gene mutation, Chinese hamster lung V-79 cells in vitro, ouabain resistance
HIM	Haemophilus influenzae, mutation
HMA	Host mediated assay, animal cells in animal hosts

Test Code	Definition
HMH	Host mediated assay, human cells in animal hosts
HMM	Host mediated assay, microbial cells in animal hosts
HSC	Hordeum species, chromosomal aberrations
HSM	Hordeum species, mutation
ICH	Inhibition of intercellular communication, human cells in vitro
ICR	Inhibition of intercellular communication, rodent cells in vitro
KPF	Klebsiella pneumonia, forward mutation
MAF	Micrococcus aureus, forward mutation
MHT	Mouse heritable translocation test
MIA	Micronucleus test, animal cells in vitro
MIH	Micronucleus test, human cells in vitro
MST	Mouse spot test
MVA	Micronucleus test, other animals in vivo
MVC	Micronucleus test, hamsters in vivo
MVH	Micronucleus test, human cells in vivo
MVM	Micronucleus test, mice in vivo
MVR	Micronucleus test, rats in vivo
NCF	Neurospora crassa, forward mutation
NCN	Neurospora crassa, aneuploidy
NCR	Neurospora crassa, reverse mutation
PLC	Plants (other), chromosomal aberrations
PLI	Plants (other), micronuclei
PLM	Plants (other), mutation
PLS	Plants (other), sister chromatid exchanges
PLU	Plants, unscheduled DNA synthesis
PRB	Prophage, induction, SOS repair, DNA strand breaks, or cross-links
PSC	Paramecium species, chromosomal aberrations
PSM	Paramecium species, mutation
RIA	DNA repair exclusive of UDS, animal cells in vitro
RIH	DNA repair exclusive of UDS, human cells in vitro
RVA	DNA repair exclusive of UDS, animal cells in vivo
SAD	Salmonella typhimurium, DNA repair-deficient strains, differential toxicity
SAF	Salmonella typhimurium, forward mutation
SAL	Salmonella typhimurium, all strains, reverse mutation
SAS	Salmonella typhimurium (other misc. strains), reverse mutation
SA0	Salmonella typhimurium TA100, reverse mutation
SA1	Salmonella typhimurium TA97, reverse mutation
SA2	Salmonella typhimurium TA102, reverse mutation
SA3	Salmonella typhimurium TA1530, reverse mutation
SA4	Salmonella typhimurium TA104, reverse mutation
SA5	Salmonella typhimurium TA1535, reverse mutation
SA7	Salmonella typhimurium TA1537, reverse mutation
SA8	Salmonella typhimurium TA1538, reverse mutation

Test Code	Definition
SA9	Salmonella typhimurium TA98, reverse mutation
SCF	Saccharomyces cerevisiae, forward mutation
SCG	Saccharomyces cerevisiae, gene conversion
SCH	Saccharomyces cerevisiae, homozygosis by recombination or gene conversion
SCN	Saccharomyces cerevisiae, aneuploidy
SCR	Saccharomyces cerevisiae, reverse mutation
SGR	Streptomyces griseoflavus, reverse mutation
SHF	Sister chromatid exchange, human fibroblasts in vitro
SHL	Sister chromatid exchange, human lymphocytes in vitro
SHT	Sister chromatid exchange, transformed human cells in vitro
SIA	Sister chromatid exchange, other animal cells in vitro
SIC	Sister chromatid exchange, Chinese hamster cells in vitro
SIH	Sister chromatid exchange, other human cells in vitro
SIM	Sister chromatid exchange, mouse cells in vitro
SIR	Sister chromatid exchange, rat cells in vitro
SIS	Sister chromatid exchange, Syrian hamster cells in vitro
SIT	Sister chromatid exchange, transformed cells in vitro
SLH	Sister chromatid exchange, human lymphocytes in vivo
SLO	Mouse specific locus test, other stages
SLP	Mouse specific locus test, postspermatogonia
SPF	Sperm morphology, F1 mouse
SPH	Sperm morphology, human
SPM	Sperm morphology, mouse
SPR	Sperm morphology, rat
SPS	Sperm morphology, sheep
SSB	Saccharomyces species, DNA breaks, cross-links or related damage
SSD	Saccharomyces cerevisiae, DNA repair-deficient strains, diff. toxicity
STF	Streptomyces coelicolor, forward mutation
STR	Streptomyces coelicolor, reverse mutation
SVA	Sister chromatid exchange, animal cells in vivo
SVH	Sister chromatid exchange, other human cells in vivo
SZD	Schizosaccharomyces pombe, DNA repair-deficient strains, diff. toxicity
SZF	Schizosaccharomyces pombe, forward mutation
SZG	Schizosaccharomyces pombe, gene conversion
SZR	Schizosaccharomyces pombe, reverse mutation
T7R	Cell transformation, SA7/rat cells
T7S	Cell transformation, SA7/Syrian hamster embryo cells
TBM	Cell transformation, BALB/C3T3 mouse cells
TCL	Cell transformation, other established cell lines
TCM	Cell transformation, C3H10T1/2 mouse cells
TCS	Cell transformation, Syrian hamster embryo cells, clonal assay
TEV	Cell transformation, other viral enhancement systems
TFS	Cell transformation, Syrian hamster embryo cells, focus assay

Test Code	Definition
TIH	Cell transformation, human cells in vitro
TPM	Cell transformation, mouse prostate cells
TRR	Cell transformation, RLV/Fischer rat embryo cells
TSC	Tradescantia species, chromosomal aberrations
TSI	Tradescantia species, micronuclei
TSM	Tradescantia species, mutation
TVI	Cell transformation, treated in vivo, scored in vitro
UBH	Unscheduled DNA synthesis, human bone-marrow cells in vivo
UHF	Unscheduled DNA synthesis, human fibroblasts in vitro
UHL	Unscheduled DNA synthesis, human lymphocytes in vitro
UHT	Unscheduled DNA synthesis, transformed human cells in vitro
UIA	Unscheduled DNA synthesis, other animal cells in vitro
UIH	Unscheduled DNA synthesis, other human cells in vitro
UPR	Unscheduled DNA synthesis, rat hepatocytes in vivo
URP	Unscheduled DNA synthesis, rat primary hepatocytes
UVA	Unscheduled DNA synthesis, other animal cells in vivo
UVC	Unscheduled DNA synthesis, hamster cells in vivo
UVH	Unscheduled DNA synthesis, other human cells in vivo
UVM	Unscheduled DNA synthesis, mouse cells in vivo
UVR	Unscheduled DNA synthesis, rat cells (other than hepatocytes) in vivo
VFC	Vicia faba, chromosomal aberrations
VFS	Vicia faba, sister chromatid exchange